

Heart Failure

Deoxyribonucleic Acid Damage/Repair Proteins Are Elevated in the Failing Human Myocardium due to Idiopathic Dilated Cardiomyopathy

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OBJECTIVES	The study investigated the expression and relationship of deoxyribonucleic acid (DNA) repair enzymes with hemodynamic and nitric oxide (NO)-mediated stress in the failing myocardium.
BACKGROUND	The role of apoptosis in human heart failure is controversial. Experimental studies suggested that NO-mediated stress modulates apoptosis of the cardiac myocytes. Of note, DNA repair enzymes such as redox factor/apurinic/apuridine endonuclease Ref-1 protein, proliferative cell nuclear antigen (PCNA), the poly (ADP-ribose) polymerase (PARP), and DNA-protein kinase (DNA-PK) determine the cell fate after the DNA damage.
METHODS	Left ventricular (LV) endomyocardial biopsies from 23 patients with dilated cardiomyopathy were analyzed by immunohistochemistry.
RESULTS	Terminal deoxynucleotidyltransferase-mediated biotin-dUTP nick-end labeling (TUNEL) or cleaved caspase-3 and cleaved PARP could not be detected. The number of Ref-1-positive myocytes tended to be higher in patients with LV ejection fraction (EF) $\leq 35\%$ versus LV EF $> 35\%$ ($21.23 \pm 4.8\%$ vs. $13.8 \pm 5.8\%$, $p = 0.1$). The PCNA ($7.1 \pm 2.8\%$ vs. $0.9 \pm 0.6\%$, $p = 0.05$) and DNA-PK expressions ($39.5 \pm 5.4\%$ vs. $8.6 \pm 5.5\%$, $p < 0.01$) were higher in patients with LVEF $\leq 35\%$ vs. LVEF $> 35\%$. The PCNA, Ref-1, and DNA-PK expression correlated with the LV end-systolic wall stress ($r = 0.61$, $p < 0.01$; $r = 0.52$, $p < 0.01$; and $r = 0.73$, $p < 0.001$, respectively). In addition, the PCNA and DNA-PK expression correlated with inducible NO synthase ($r = 0.41$, $p = 0.05$, and $r = 0.53$, $p < 0.01$, respectively).
CONCLUSIONS	In this study, apoptosis could not be detected in the failing myocardium owing to idiopathic dilated cardiomyopathy. In contrast, failing myocardium was characterized by active DNA repair that was associated with elevated LV wall stress and activation of the inducible NO synthase. (J Am Coll Cardiol 2002;40:1097-103) © 2002 by the American College of Cardiology Foundation

Apoptosis is a cell death pathway whose role in human heart failure is provocative but as yet unproven (1-6). Previous studies reporting a wide range of apoptosis (1-6) were performed in explanted end-stage failing hearts and based on the terminal deoxynucleotidyltransferase-mediated biotin-dUTP nick-end labeling (TUNEL) technique to

(11,12) or toward the repair of the damaged DNA (13-18). Downregulation of the redox factor apurinic/apuridine endonuclease Ref-1-protein (APE/Ref-1) is consistent with the DNA repair failure and was shown to coincide with apoptosis in several cell types (11,12). The proliferative cell nuclear antigen (PCNA) (13,14) and poly (adenosine diphosphate-ribose) polymerase (PARP) (15,16) are other proteins required for a successful nucleotide excision repair. In addition, the catalytic subunit of the DNA-dependent protein kinase (DNA-PKs) is a key signaling protein sensing the DNA damage and triggering the cascade of a DNA double-strand repair (17,18). Repair of cardiac myocytes could be an important defensive mechanism of the failing heart to maintain normal left ventricular (LV) wall stress by preserving the number of the myocytes. However, the expression of these enzymes and their relationship with hemodynamic phenotype in human heart failure is not known. In addition, the inducible nitric oxide synthase (iNOS) and its mediated oxidative stress have been postulated to modulate LV contractile function in vivo. Nevertheless, the effects of iNOS on the nuclear repair or cell

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detect deoxyribonucleic acid (DNA) fragmentation as a marker of apoptosis (5,6). However, the accuracy of this technique can be confounded by living cells undergoing ribonucleic acid (RNA) synthesis/splicing (7), active DNA repair/proliferation (8-10), or by methodologic set-up (8-10).

Of note, several DNA repair enzymes determine the fate of the cell after DNA damage either toward apoptosis

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Abbreviations and Acronyms

APE/Ref-1	= redox factor apurinic/apyrimidine endonuclease Ref-1 protein
DNA	= deoxyribonucleic acid
DNA-PKcs	= deoxyribonucleic acid-protein kinase catalytic subunit
iNOS	= inducible nitric oxide synthase
LV	= left ventricle/ventricular
LVEF	= left ventricular ejection fraction
PARP	= poly (adenosine diphosphate-ribose) polymerase
PCNA	= proliferative cell nuclear antigen
RNA	= ribonucleic acid
TUNEL	= terminal deoxynucleotidyltransferase-mediated biotin-dUTP nick-end labeling

death are controversial (19–22). Therefore, this study tested the hypothesis that the failing myocardium due to idiopathic dilated cardiomyopathy undergoes active DNA repair and that this reparative process is related to both hemodynamic and nitric oxide-mediated stress.

METHODS

Patients. Twenty-six patients undergoing left and right heart catheterization and LV biopsy due to idiopathic dilated cardiomyopathy were selected. No patient had significant coronary artery diseases as documented by angiography. All patients received standard heart failure medication including diuretics and angiotensin-converting enzyme inhibitors. Only three patients received beta-blockers prior to catheterization. None of the patients received inotropic or other intravenous therapy. In addition, none of the patients underwent any other invasive procedure or suffered from the surinfection prior to catheterization. The biopsies were fixed in 4% paraformaldehyde for further analyses. Of the total number of biopsies, two were too small to be analyzed, and in one biopsy several stainings failed, presumably due to insufficient fixation. The study protocol was approved by the local ethical committee.

TUNEL labeling. The TUNEL labeling was performed using “the stringent approach” without pretreatment with proteinase K as previously described (7–9). Negative controls included omission of terminal deoxynucleotidyl transferase (TdT) from the labeling mixture. Tonsils were used as positive controls.

Immunohistochemistry. To analyze the degree of interstitial inflammation, the leukocytes in the interstitial regions were detected using the CD34 antibody (concentration 1/400, Dako, Copenhagen, Denmark). To estimate the extent of fibrosis in the LV biopsies a trichrome-Masson staining was used. To detect the cleavage of proteins during apoptosis, the biopsies were stained with anti-cleaved caspase-3 antibody (concentration 1/200) and cleaved PARP antibody (concentration 1/750, Pharmingen, San Diego, California). To investigate the proteins involved in the DNA repair/synthesis, specimens were stained with

antibodies against PARP (concentration 1/750), PCNA (concentration 1/30, Dako, Copenhagen, Denmark), APE/Ref-1 protein (concentration 1/400, Santa Cruz, CA), DNA-PKcs (concentration 1/100, Neomarkers/Labovision), and Ki67 (Dako). The iNOS antibody (concentration 1/2,500, Biomol) was used to detect the iNOS protein. Finally, 8-oxo-deoxyguanosine staining (concentration 1/800, The Japan Institute for the Control of Aging) was performed to detect the presence of oxidative DNA damage. All primary antibodies were detected by a peroxidase antibody conjugate technique using either a goat anti-mouse peroxidase antibody (Jackson Laboratories) or rabbit anti-rat antibodies (Dako, Copenhagen, Denmark). Tonsils and atherosclerotic plaques were used as controls.

Histological quantification. The quantifications were performed using a point-counting grid. The extent of fibrosis was quantified using a point-counting grid from the entire biopsy. The LV myocyte diameter was averaged from myocytes sectioned perpendicular to the direction of the nucleus. The extent of PCNA, DNA-PKcs, and APE/Ref-1 protein-labeled myocytes was analyzed by a systematic random sampling as previously described (23,24) by counting 500 to 750 cells in 15 fields. Among the total number of cells, 250 to 300 myocytes were counted. The extent of the CD34, iNOS, 8-oxo-deoxyguanosine, and PARP staining was analyzed semiquantitatively on a 1-to-4 scale based on the number of stained cells and on the intensity of the staining. All histological quantitative analyses were performed by observers unaware of the hemodynamic data.

Hemodynamic analysis. The left ventricular ejection fraction (LVEF) was derived from the single-plane angiogram using the area-length method. Patients were divided into two groups according to their ejection fraction. Patients with LVEF $\leq 35\%$ were considered to have severe heart failure. Both LV systolic and diastolic wall stress levels were calculated using the combined echocardiographic and invasive hemodynamic data ($n = 16$).

Statistical analysis. All results are given as mean \pm SEM. The Student *t* test, Fisher exact test, and Spearman correlation coefficient and liner regression analyses were used for appropriate comparisons. A *p* value of <0.05 was considered statistically significant.

RESULTS

Hemodynamics. Individual hemodynamic data and relative expression of DNA repair markers are shown in Table 1. Hemodynamic characteristics in both groups are shown in Table 2. Patients with LVEF $\leq 35\%$ had larger LV volumes, higher LV end-diastolic pressure, and higher LV systolic and diastolic wall stress as compared to patients with LVEF $>35\%$. In addition, LV developed pressure tended to be lower in patients with low LVEF.

Morphological analysis. Patients with LVEF $\leq 35\%$ had larger extent of interstitial fibrosis ($23.8 \pm 5.5\%$ vs. $7.6 \pm$

Table 1. Individual Patient Characteristics

Patient #	Gender	Age (yrs)	LVEF (%)	LV devP (mm Hg)	LV EDP (mm Hg)	PCNA (%)	DNA-PK (%)	APE/Ref-1 (%)
1	F	35	57	133	16	0	0	4.87
2	M	51	57	173	18	0	31.43	26.83
3	M	45	54	111	7	4.61	0	40.74
4	F	69	52	82	18	2.54	0.54	1.17
5	F	64	51	174	14	0	36.21	30.95
6	F	55	49	110	22	0	0	3.95
7	F	64	45	126	24	0	0	1.09
8	M	59	43	134	6	0.14	0.79	0.71
9	F	65	35	124	26	1.81	41.30	12.35
10	M	49	35	88	22	0	36.21	3.61
11	M	46	33	121	9	33.05	77.27	19.51
12	F	29	28	142	27	3.37	48.56	38.18
13	M	57	26	123	27	25.81	39.47	73.07
14	M	56	23	124	10	0	21.62	1.32
15	M	48	22	80	22	0	57.89	13.69
16	M	38	22	126	12	0	14.81	8.33
17	F	48	20	115	24	4.87	44.44	22.78
18	F	43	18	84	28	0	0	5.31
19	M	66	18	83	17	0	58.33	16.67
20	M	57	36	109	23	8.91	29.87	34.41
21	M	45	23	138	22	2.78	48.67	21.12
22	M	59	13	93	30	24.71	61.24	48.05
23	M	58	28	94	27	1.79	12.18	20.63

APE/Ref-1 = redox factor apurinic/aprimidine endonuclease Ref-1 protein; DNA-PK = deoxyribonucleic acid-protein kinase; LVEF = left ventricular ejection fraction; LV devP = left ventricular developed pressure; LV EDP = left ventricular end-diastolic pressure; PCNA = proliferative cell nuclear antigen.

1.7%, $p < 0.01$) and higher incidence of CD34-positive cells (63% [10/16] vs. 25% [2/8], $p < 0.05$) as compared to patients with LVEF $< 35\%$. Both groups had similar degree of cellular hypertrophy as assessed by the LV short-axis diameter ($28.6 \pm 1.4 \mu\text{m}$ vs. $25.6 \pm 1.8 \mu\text{m}$, $p = \text{NS}$).

TUNEL labeling. In this study, no positive TUNEL labeling could be detected in any patient regardless of the degree of LV dysfunction (Fig. 1). In addition, no positive staining for cleaved PARP or cleaved caspase-3 in the LV biopsies of any patient could be detected. These observations argue against the presence of apoptosis in our patients with idiopathic cardiomyopathy.

DNA repair enzymes. Figure 2 and Table 3 show the percentage of the cardiac myocytes expressing the DNA repair/synthesis enzymes. The APE/Ref-1 protein was found in all patients, and the number of Ref-1 protein-positive myocytes tended to be higher in patients with

LVEF $> 35\%$ as compared to patients with LVEF $\leq 35\%$. In addition, the incidence of the PARP expression was similar in patients with LVEF $\leq 35\%$ as compared to patients with LVEF $> 35\%$ (99% [7/8] vs. 67% [10/15], $p = \text{NS}$). Second, positive PCNA staining was observed in 3 patients (37%) with LVEF $> 35\%$ and in 10 patients (67%) with LVEF $\leq 35\%$ ($p = \text{NS}$). The number of PCNA-positive myocytes was higher in patients with LVEF $\leq 35\%$. Finally, the expression of DNA-PKcs was found in 4 patients with LVEF $> 35\%$ (50%) and in 14 patients with LVEF $\leq 35\%$ (93%, $p = 0.05$). The number of the DNA-PKcs-positive cardiac myocytes was higher in patients with LVEF $\leq 35\%$ as compared to patients with LVEF $> 35\%$. Furthermore, a weak but significant correlation was observed between the DNA-PKcs and PCNA ($r = 0.54$, $p = 0.008$) and APE/Ref-1 protein expression ($r = 0.43$, $p = 0.04$).

To investigate whether activation of the PCNA and DNA-PKcs proteins is associated with the cell proliferation, LV endomyocardial biopsies were stained for the Ki-67 protein, a marker of DNA replication. No biopsy specimen stained positive for this protein, suggesting that the PCNA activation was due to the DNA repair.

Hemodynamic and humoral correlates of the nuclear repair. Figure 3 shows the correlation between the hemodynamic indices and myocardial DNA-PKcs. A significant relationship was found between the LV systolic wall stress and expression of the PCNA, APE/Ref-1 protein, and DNA-PKcs. Furthermore, a significant inverse relationship

Table 2. Hemodynamics

	LVEF $> 35\%$ (n = 8)	LVEF $\leq 35\%$ (n = 15)
LVEF (%)	51 ± 5	$25 \pm 7^*$
LV EDVI (ml/m ²)	77 ± 28	$130 \pm 52^*$
LV ESVI (ml/m ²)	29 ± 10	$86 \pm 36^*$
LV devP (mm Hg)	130 ± 31	112 ± 25
LV EDP (mm Hg)	16 ± 6	$22 \pm 7^*$
LV systolic wall stress (kdyn/cm ²)	112 ± 20	$194 \pm 54^*$
LV diastolic wall stress (kdyn/cm ²)	28 ± 16	$46 \pm 18^*$

* $p < 0.01$ vs. LVEF $> 35\%$.

LV = left ventricular; LV EDVI = left ventricular end-diastolic volume index; LV ESVI = left ventricular end-systolic volume index; Other abbreviations as in Table 1.

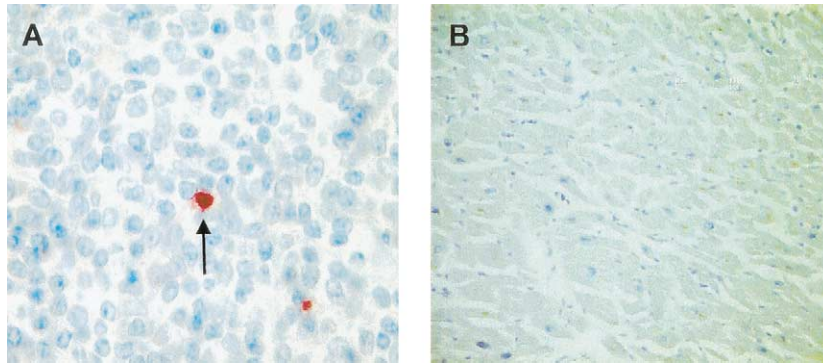


Figure 1. An example of “stringent” TUNEL without the use of proteinase K in positive control (tonsil **panel A**, $\times 1000$) and in a patient with dilated cardiomyopathy (**panel B**, $\times 200$). Arrows indicate the presence of TUNEL-positive nuclei in tonsils. Note that when utilizing the “stringent” approach, no TUNEL-positive labeling has been observed in dilated cardiomyopathy patients.

was observed between the number of DNA-PKcs-positive myocytes and LVEF ($r = -0.53$, $p = 0.003$). Finally, the number of DNA-PKcs-positive myocytes correlated significantly with both LV end-diastolic and end-systolic volume indices ($r = 0.59$, $p = 0.008$, and $r = 0.57$, $p = 0.01$).

To investigate whether the DNA repair process is associated with the oxidative DNA damage, LV biopsies were analyzed for the iNOS and 8-oxo-deoxyguanosine. The expression of DNA-PKcs and PCNA correlated significantly with the iNOS expression in the cardiac myocytes ($r = 0.53$, $p = 0.02$, and $r = 0.41$, $p = 0.05$, respectively). As shown in Figure 4, there was a good concordance in the iNOS expression and the DNA-PKcs expression in the entire study population and in patients with LVEF $\leq 35\%$. Of note, 8-oxo-deoxyguanosine, a marker of oxidative DNA damage, was found in 62% (5/8) of patients with LVEF $> 35\%$ and in 73% (11/15) of patients with LVEF $\leq 35\%$ (NS). Furthermore, the Ref-1 protein and PCNA expression correlated significantly with 8-oxo-deoxyguanosine expression ($r = 0.64$, $p = 0.01$, and $r = 0.48$, $p = 0.02$).

DISCUSSION

The results of our study show that: 1) cardiac myocytes of patients with LV dysfunction do not show TUNEL-positive labeling or cleavage of proteins during the process of apoptosis regardless of the severity of heart failure; and 2) in contrast, idiopathic cardiomyopathy is associated with active DNA repair that appears to be associated with elevated LV wall stress and activated iNOS.

Apoptosis in human heart failure. The prevalence of apoptosis in patients with end-stage heart failure varies widely (1–6). In contrast to previous studies (1–6), we did not observe TUNEL-positive labeling or other apoptotic markers such as cleaved caspase-3 or cleaved PARP in any LV endomyocardial specimen either in severe or mild LV dysfunction. Our data are consistent with Kanoh et al. (10), who despite positive TUNEL staining also failed to observe other morphological markers of apoptosis in patients with dilated cardiomyopathy. Likewise, other studies reported lower or no incidence of positive TUNEL staining in end-stage myopathies (3,4,9).

A sharp difference in the prevalence of apoptosis may be due to several reasons. First, previous studies (4,5) uniformly included patients with end-stage heart failure, and detection of apoptosis was performed in explanted hearts. In contrast, we investigated LV endomyocardial biopsies obtained in vivo and studied patients with a wide range of LV dysfunction. Second, previous studies utilized conventional TUNEL assay with proteinase K pretreatment. This may cause interference with the RNA synthesis or DNA repair in the living cells (7). To avoid such false positive labeling, we utilized a “stringent” TUNEL technique without pretreatment with proteinase K that avoids a specific binding due to DNA repair or necrosis (7–9).

DNA repair in human heart failure. In contrast to cell death, several findings in our study suggest that failing cardiac myocytes were undergoing active DNA repair. First, the expression of the APE/Ref-1 protein was preserved in

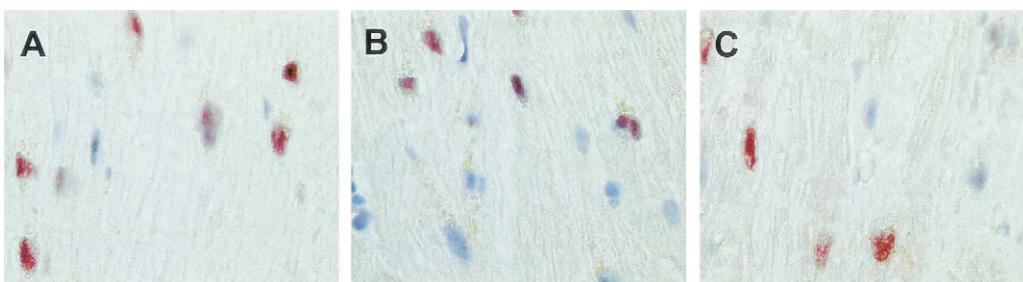


Figure 2. An example of staining for the deoxyribonucleic acid-protein kinase catalytic subunit (A), proliferative cell nuclear antigen (B), and apurinic/aprimidine endonuclease/redox factor 1 (C) from a patient with left ventricular ejection fraction $\leq 35\%$ ($\times 1000$ magnification).

Table 3. DNA Repair Enzymes

	LVEF >35% (n = 8)	LVEF ≤35% (n = 15)
APE/Ref-1-positive myocytes (%)	13.8 ± 5.7	21.2 ± 4.8
PCNA-positive myocytes (%)	0.9 ± 1.6	7.1 ± 2.8*
DNA-PKcs-positive myocytes (%)	8.6 ± 5.5	39.5 ± 5.4*

*p < 0.05 vs. LVEF >35%.
APE/Ref-1 = the redox factor/apurinic/aprimidine endonuclease Ref-1 protein;
DNA-PKcs = catalytic subunit of the DNA protein kinase; LVEF = left ventricular
ejection fraction; PCNA = proliferating cell nuclear antigen.

all patients. This DNA enzyme is responsible for preserving the genomic stability by repairing the apurinic/aprimidinic sites, and its downregulation appears to precede the cell death during the neuronal apoptosis (11,12). Our data corroborate the hypothesis that the preserved expression of this base excision-repair enzyme in advanced heart failure may be crucial to prevent apoptosis in nonischemic cardiomyopathy. Second, the expression of the PCNA protein, a co-factor of DNA polymerase required for DNA synthesis (13,14), was higher in patients with severe LV dysfunction. Third, the DNA-PKcs is a crucial component of the DNA double-strand break-repair machinery that recognizes DNA damage and recruits the DNA repair machinery (17,18,25). Of note, the enzyme is upregulated during the cell division (26) or during nonhomologous recombination and repair (27). In this study, we report a novel observation of upregulation of the DNA-PKcs in cardiac myocytes of the failing myocardium. Because no LV biopsy specimen showed the expression of the Ki-67 protein (28), the activation of both PCNA and DNA-PKcs in the failing myocytes corroborates the presence of active nuclear excision repair rather than DNA replication (10). Finally, none of the LV endomyocardial specimen showed the cleaved form of PARP. In contrast, the PARP protein was detected in the majority of the patients with severe LV dysfunction. Thus, these data indicate that the failing human myocardium due to idiopathic dilated cardiomyopathy is associated with DNA damage. Moreover, our observations support the hypothesis that, in response to the DNA damage, failing

All patients (N=23)			LVEF < 35% (N=15)		
	iNOS+	iNOS-		iNOS+	iNOS-
DNA PK +	84% (11)	60% (6)	DNA PK +	100% (11)	75% (3)
DNA PK -	16% (2)	40% (4)	DNA PK -	0% (0)	25% (1)
Concordance = 0.65			Concordance = 0.80		

Figure 4. Relationship between the deoxyribonucleic acid-protein kinase catalytic subunit (DNA-PKcs) and inducible nitric oxide synthase (iNOS) expressions in all patients and in patients with left ventricular ejection fraction (LVEF) ≤35%.

cardiac myocytes primarily recruit factors aimed for cell survival rather than for cell death.

Hemodynamic and humoral factors associated with DNA damage/repair. The factors and mechanisms associated with the DNA damage/repair in human heart failure are not entirely clear. Our observation of upregulated DNA-PKcs and other DNA repair enzymes in patients with elevated LV wall stress suggests that pure mechanical load is sufficient to trigger the DNA damage/repair process. This corroborates earlier in vitro observations of a direct myocyte damage in response to acute mechanical stretch (29). However, in contrast to this study, the failure to observe markers of apoptosis in our study suggests that the cellular response to the mechanical load in vivo is complex and may depend on the local environment.

In this regard, recent experimental study indicated that pacing-induced heart failure is associated with increased levels of nitrotyrosine and oxidative stress that shift the balance from DNA repair toward apoptosis with loss of DNA-PKcs expression (22). In our study, 8-oxo-deoxyguanosine, a marker of oxidative DNA damage, was detected in the cardiac myocytes of the majority of patients, even those with severe LV dysfunction. However, in contrast to the latter experimental study, all patients but one stained positive for DNA-PKcs. In addition, the marker of

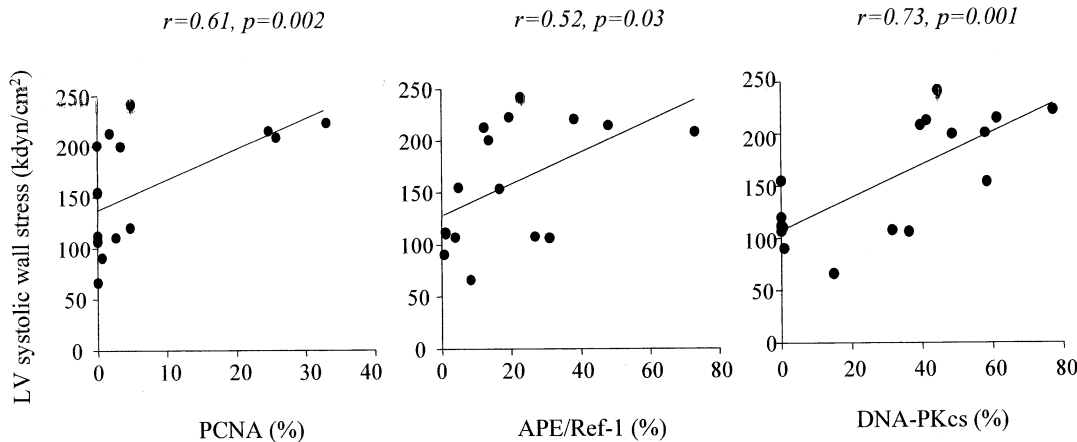


Figure 3. Relationship between the proliferative cell nuclear antigen (PCNA), apurinic/aprimidine endonuclease/redox factor 1 (APE/Ref-1), and deoxyribonucleic acid-protein kinase catalytic subunit (DNA-PKcs) expression and left ventricular (LV) systolic wall stress.

oxidative nuclear damage correlated with other DNA repair proteins. This suggests that, in the failing myocardium, even a high degree of oxidative DNA damage is followed primarily by increased DNA repair activity, and apoptosis occurs only after defensive mechanisms fail (30).

Of note, previous studies suggested that iNOS-mediated signaling may play a dual role in cellular defensive mechanisms.

On the one hand, *in vitro* studies demonstrated that iNOS-mediated oxidative stress is an important regulator of apoptosis (19,20). On the other hand, it has been also shown that NO-mediated signaling may inhibit caspase activity (21). In addition, a recent study indicated that NO interacts with the DNA repair machinery by activating the DNA-PKcs (31). In the present study, we observed positive iNOS staining in the majority of patients with advanced heart failure, and, yet, no activation of caspases was seen. In contrast, the presence of iNOS activation coincided with the expression of markers of DNA repair enzymes such as PCNA and DNA-PKcs in patients with severely depressed LV function. Thus, our observations corroborate the hypothesis that local cardiac excessive NO generation *in vivo* does not inevitably cause cell death. Conversely, it can promote the DNA repair/survival machinery by the mechanisms that are not entirely clear from the present study. Taken together, these data suggest that DNA damage in the failing myocardium results from a direct hemodynamic stress where humoral factors are likely to play a modulatory role.

Study limitations. First, LV endomyocardial biopsies though taken *in vivo* represent only a small part of the LV, and absence of apoptotic markers in the biopsy does not exclude the possibility of apoptosis in other parts of the LV. Second, apoptosis and repair are dynamic processes, and one-time analysis may underestimate the incidence of apoptosis or repair. Furthermore, apoptosis was assessed using only the “stringent” TUNEL technique and from the cleavage of caspase-3 and PARP, and no other techniques such as Taq polymerase assay or electromicroscopy were performed to detect apoptotic cell death. In addition, we did not perform other RNA or protein lysate analyses to quantitate the expression of DNA repair enzymes in the LV endomyocardial biopsies. Finally, we studied only patients with dilated cardiomyopathy, and thus our results cannot be extended to other models of LV dysfunction. In particular, previous studies demonstrated that ischemia and ischemia/reperfusion injury are potential apoptotic stimuli (32,33). Moreover, in the pressure-overload hypertrophy model (34,35), the presence of apoptosis coincides with the transition from compensated hypertrophy to heart failure.

Conclusions. The present study supports the hypothesis that, in nonischemic dilated cardiomyopathy, the incidence of apoptosis is low and does not substantially contribute to the progression of disease. In contrast, advanced heart failure is associated with the activation of DNA repair proteins. This appears to be associated with elevated LV wall stress and iNOS. Further studies are required to

investigate the mechanisms contributing to the induction of nuclear reparative processes rather than to mechanisms contributing to cell death.

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REFERENCES

- Haunstetter A, Izumo S. Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circ Res* 1998;82:1111–29.
- Kang PM, Izumo S. Apoptosis and heart failure. A critical review of the literature. *Circ Res* 2000;86:1107–13.
- Schaper J, Elsässer A, Kostin S. The role of cell death in heart failure. *Circ Res* 1999;85:867–9.
- Elsässer A, Suzuki K, Lorenz-Meyer S, et al. The role of apoptosis in myocardial ischemia: a critical appraisal. *Basic Res Cardiol* 2001;96: 219–26.
- Narula J, Haider N, Virmani R, et al. Apoptosis in myocytes in end-stage heart failure. *N Engl J Med* 1996;335:1182–9.
- Olivetti G, Abbi R, Quaini F, et al. Apoptosis in the failing human heart. *N Engl J Med* 1997;336:1131–41.
- Kockx MM, Muhring J, Knaapen MWM, et al. RNA synthesis and splicing interferes with DNA *in situ* end labeling techniques to detect apoptosis. *Am J Pathol* 1998;152:886–8.
- Kockx MM, Knaapen MWM. The role of apoptosis in vascular disease. *J Pathol* 2000;190:267–80.
- Knaapen MWM, Davies MJ, De Bie M, et al. Apoptotic versus autophagic cell death in heart failure. *Cardiovasc Res* 2001;51:304–12.
- Kanoh M, Takemura G, Misao J, et al. Significance of myocytes with positive DNA *in situ* nick end-labeling (TUNEL) in hearts with dilated cardiomyopathy: not apoptosis but DNA repair. *Circulation* 1999;99:2757–64.
- Kawase M, Fujimura M, Morita-Fujimura YM, Chan PH. Reduction of apurinic/apyrimidinic endonuclease expression after transient global cerebral ischemia in rats: implications of the failure of DNA repair in neuronal apoptosis. *Stroke* 1999;30:441–8.
- Robertson KA, Hill DP, Xu Y, et al. Down-regulation of apurinic/apyrimidinic endonuclease expression is associated with the induction of apoptosis in differentiating myeloid leukemia cells. *Cell Growth Differ* 1997;8:443–9.
- Shivji KK, Kenny MK, Wood RD. Proliferating cell nuclear antigen is required for DNA excision repair. *Cell* 1992;69:367–74.
- Savio M, Stivala LA, Bianchi L, et al. Involvement of the proliferating cell nuclear antigen (PCNA) in DNA repair induced by alkylating agents and oxidative damage in human fibroblasts. *Carcinogenesis* 1998;19:591–6.
- De Murcia G, Menissier-de Murcia J. Poly(ADP-ribose) polymerase: a molecular nick-sensor. *Trends Biochem Sci* 1994;19:172–6.
- Althaus FR, Richter C. ADP ribosylation of proteins. Enzymology and biological significance. *Mol Biol Biochem Biophys* 1987;37:1–237.
- Featherstone C, Jackson SP. DNA-dependent protein kinase gets a break: its role in repairing DNA and maintaining genomic integrity. *Br J Cancer* 1999;80 Suppl 1:14–9.
- Smith GCM, Jackson SP. The DNA-dependent protein kinase. *Genes Dev* 1999;13:916–34.
- Arstall MA, Sawyer DB, Fukazawa R, et al. Cytokine-mediated apoptosis in cardiac myocytes: the role of inducible nitric oxide synthase induction and peroxynitrite generation. *Circ Res* 1999;85: 829–40.
- Ing DJ, Zang J, Dzau VJ, et al. Modulation of cytokine-induced cardiac myocyte apoptosis by nitric oxide, Bak, and Bcl-x. *Circ Res* 1999;84:21–3.
- Kim YM, Bombeck CA, Billiar TR. Nitric oxide as a bifunctional regulator of apoptosis. *Circ Res* 1999;84:253–6.
- Cesselli D, Jakoniuk I, Barlucchi L, et al. Oxidative stress-mediated cardiac cell death is a major determinant of ventricular dysfunction and failure in dog dilated cardiomyopathy. *Circ Res* 2001;89:279–86.

23. Cruz-Orive LM, Weibel ER. Sampling designs for stereology. *J Microsc* 1980;122:235-57.
24. Gundersen HJG, Osterby R. Optimizing sampling efficiency of stereological studies in biology: or "do more less well." *J Microsc* 1980;121:65-73.
25. Casciola-Rosen LA, Anhalt GJ, Rosen A. DNA-dependent protein kinase is one of a subset of autoantigens specifically cleaved early during apoptosis. *J Exp Med* 1995;182:1625-34.
26. Lee SE, Mitchell RA, Cheng A, et al. Evidence for DNA-PK-dependent and -independent DNA double-strand break repair pathways in mammalian cells as a function of the cell cycle. *Mol Cell Biol* 1997;17:1425-33.
27. Grawunder U, Finnie N, Jackson SP, et al. Expression of DNA-dependent protein kinase holoenzyme upon induction of lymphocyte differentiation and V(D)J recombination. *Eur J Biochem* 1996;241: 931-40.
28. Sawhney N, Hall PA. Ki-67: structure, function and new antibodies. *J Pathol* 1992;168:161-2.
29. Cheng W, Li B, Kajstura J, et al. Stretch-induced programmed myocyte cell death. *J Clin Invest* 1995;96:2247-59.
30. Martinet W, Knaapen MWM, De Meyer GRY, et al. Oxidative DNA damage and repair in experimental atherosclerosis are reversed by dietary lipid lowering. *Circ Res* 2001;88:733-9.
31. Xu W, Liu L, Smith GCM, et al. Nitric oxide upregulates expression of DNA-PKcs to protect cells from DNA-damaging anti-tumour agents. *Nat Cell Biol* 2000;2:339-45.
32. Yaoita H, Ogawa K, Maehara K, et al. Apoptosis in relevant clinical situations: contribution of apoptosis in myocardial infarction. *Cardiovasc Res* 2000;45:630-41.
33. Zhao ZQ, Nakamura M, Wang NP, et al. Reperfusion induces myocardial apoptotic cell death. *Cardiovasc Res* 2000;45:651-60.
34. Ding B, Price RL, Goldsmith EC, et al. Left ventricular hypertrophy in ascending aortic stenosis mice: anoikis and the progression to early failure. *Circulation* 2000;101:2854-62.
35. Teiger E, Than VD, Richard L, et al. Apoptosis in pressure overload-induced heart hypertrophy in the rat. *J Clin Invest* 1996;97:2891-7.